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Synchronization of *Oryza sativa* L.cv. Taipei-309 cell suspension culture

Ágnes Lendvai*, Krisztina Nikovics, László Bakó, Dénes Dudits, János Györgyey

Institute of Plant Biology, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary

ABSTRACT Synchronous cell systems are among the most suitable models to study the plant cell cycle. However, to date, the tobacco BY-2 cell line is the plant cell line, which can be synchronized to high levels. The aim of the present work is to produce a fast growing rice cell suspensions and to elaborate the synchronization method of the rice suspension culture. A cell synchrony starting at late G1 and S phase is obtained after release of rice cells from hydroxyurea treatment. Using this synchronous system it has been possible to demonstrate the cell cycle-dependent oscillation of transcript level of several genes, such as cullin. Furthermore, this system has facilitated the structural and biochemical analysis of cell cycle specific occurrences (the development of phragmoplast and the formation of microtubules).

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KEY WORDS

hydroxyurea
cell cycle
Oryza sativa
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The animals and plants differ both in their general strategy of ontogenesis and in environmental adaptation. Due to the presence of cell wall and the sessile lifestyle of plants, motility and cell migration cannot be used during the developmental processes and in response to environmental changes. Dedifferentiation and subsequent differentiation of plant cells require mitotic reactivation, which may result in formation of new cell types and organs, which fulfill the required function in response to the environmental stimuli. Flexible and, at the same time, precise regulation of the mitotic activity is an important prerequisite of the above response.

The cell division is composed of the replication of genetic materials (S phase) and the successive distribution of genetic materials as well as the other cell components to the two daughter cells (M phase). The recurrent progression of the above processes intermitted by two gap phases (G1 and G2) defined as the cell cycle, which is a fundamental subject in current cell biology. Although the first recognition of cell cycle was proposed in studies on plant cells (Howard and Pelc 1951), understanding of molecular events of the cell cycle is far behind that of animal cells and that of yeast (Baserga 1985). This is due partly to the lack of a suitable cell synchronization systems in plant cells.

The studies of the last dozen years revealed that the essential mechanisms of the cell cycle are common among all eukaryotic organisms. However plant-specific features of the cell cycle have been already demonstrated, *e.g.* the presence of G2/M phase specific cyclin-dependent kinases (Magyar et al. 1997), which have not been found in animals and yeasts yet. Therefore, further plant research on this field might provide more detailed insight into fundamental cellular process.

Materials and Methods

The rice-Taipei 309 cell line was growing in AA medium in the presence of 2 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D). The suspensions were subcultured every week; the approximate doubling time is 5 days.

For the synchronizations we use 2-day-old cells (4 gram cells in 100 ml culture volume) in amino acid medium (AA medium), supplemented with hydroxyurea (10 mM), which has been added freshly before use. The cells were incubated with the blocking reagent for 24 hours. After the hydroxyurea treatment we washed the cells with conditional medium. We collected the samples every 3 hours for mitotic index (MI), RNA extraction and flow cytometric analysis. Mitotic index was counted in fluorescence microscope. The cells were fixed in PBS, which contains 4% paraformaldehyde. For nuclei isolation we used the buffer of Galbraith et al. (1983). The fixed intact nuclei were stained by ethidium bromide and were analyzed by Becton & Dickinson FACS Calibur (Galbraith et al. 1989).

Total cellular RNAs were prepared from the synchronised rice cells. Equivalent amounts of total RNAs were separated on agarose-formaldehyde gels and transferred to nylon filters. Filters were hybridised overnight at 42ºC either with labelled full-length rice Skp probe or with a labelled cullin probe of

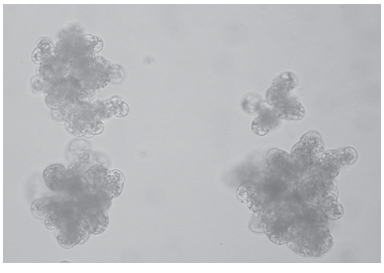

Figure1. Cell suspension of the Taipei-T309 line.

*Corresponding author. E-mail: Novodiff@nucleus.szbk.u-szeged.hu

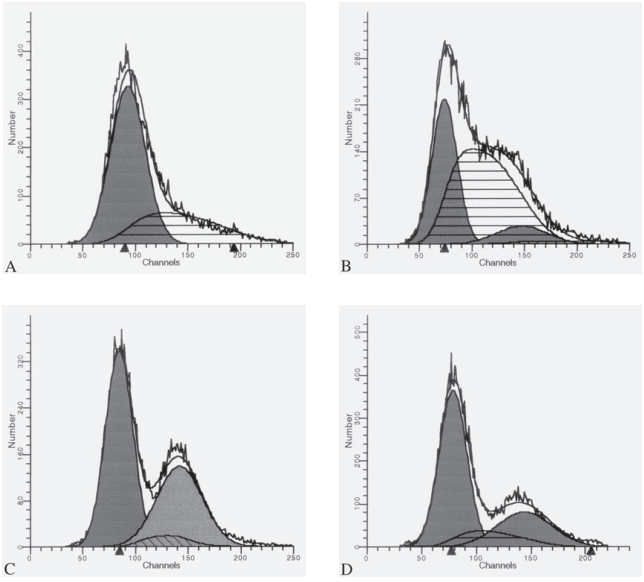

Figure 2. Flow cytometric measurements were analysed by ModFit software: A, After washing the blocking reagent B, C, D, 6, 9, 21 hours after washing, respectively.

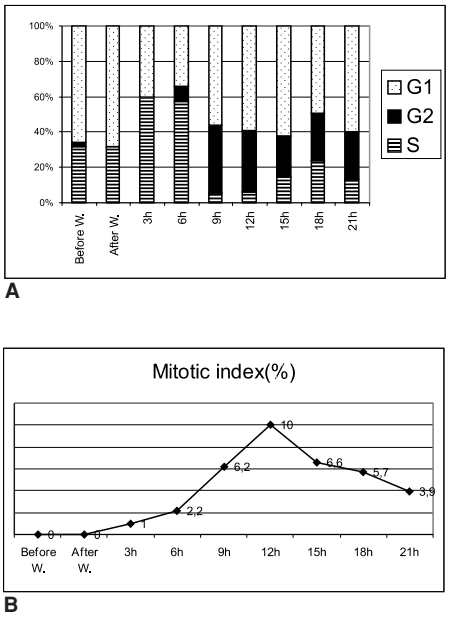

Figure 3. Distribution of cells in different cell cycle phases after release from HU block. A, The frequency of cells in various cell cycle phases was determined both by flow cytometry (G2, S, G1) and B, by counting the mitotic index.

460bp-length derived from the 5'-end of the cullin cDNA. After the final washing steps (1XSSC at 65ºC for the Skp and 0.1XSSPE at 65ºC for the cullin probe) filters were evaluated by Phosphor Imager scanning.

Result and Discussion

Improving the rice cell suspension

Cell suspension of Taipei-309 line was established in AA medium in the presence of 2,4-D. The small cell cluster-containing fraction of the suspension was transferred into new medium systemically at every subculture. As a result of the continuous selection a suspension has been obtained that is fairly homogenous, rapidly growing and fine.

Synchronization of rice suspension

Both the flow cytometric analysis and determination of mitotic index indicate that partial synchrony was achieved by the 10 mM hydroxyurea (HU) block. About 70% of the cells were blocked in G1 phase and about 30% could enter into S phase (Fig. 3A). Negligible portion was in G2 phase at the time of removal of the blocking agent. In 3 hours, the majority (app. 60%) of the cells were in S phase indicating synchronous progression of the cell cycle. Sharp increase in the ratio of the G2 cells at 9-12 hours (35-40%) was observed. In accordance with the appearance of the G2 cells, the peak of the mitotic index appeared at 12 hours (Fig. 3B).

A, The frequency of cells in various cell cycle phases was determined both by flow cytometry (G2, S, G1) and B, by counting the mitotic index

From the time cells were released from the HU arrest, the amount of Skp transcript increased steadily and reached a highest level in mitosis (G2/M maximum was observed at 18h.). On the contrary, there was no significant alteration in the cullin transcript levels (Fig. 4).

Further optimization of the synchronization method is needed to improve the synchrony of the culture, however the above describe model system is already suitable to test the expression profile of cell cycle regulated genes isolated or to be cloned in the future. This improving rice cell synchronization system may become a monocot alternative of the dicot BY-2 system.

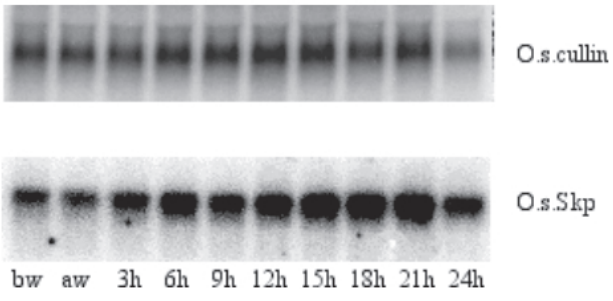

Figure 4. Northern analysis of cullin and Skp transcript levels in rice cells synchronized by hydroxyurea treatment.

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